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Callus Cell Formation and Suspension Culture of *Typha latifolia*.

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Propagation and Tissue Culture

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Abstract. This study is the first reported attempt to generate a growth curve from *Typha latifolia* L. (broadleaf cattail) callus cells in suspension culture. Several media and hormone combinations were tested for their capacity to induce callus cell formation from *T. latifolia* leaf sections and both male and female inflorescence spikes. A *T. latifolia* callus cell line was successfully established from immature female inflorescence spikes. Callus growth on Gamborgs B5 medium supplemented with 5 mg●L^{-1} dicamba and 1 mg●L^{-1} BA was superior to other media examined. A growth curve in suspension culture was generated on the most favorable culture medium for callus growth. The mass of the cells increased by 150% by the end of the growth curve. Chemical names used: dicamba (3,6-dichloro-2-methoxybenzoic acid); N^6-benzyladenine (BA).

Typha latifolia (broadleaf cattail) L. is a native perennial herb that can grow up to10 ft in height, and forms flowers from June through August. It grows from thick, underground rhizomes that survive extreme winters and produce shoots in the spring. This plant can produce more than 2,000 g●m^{-2}●$year^{-1}$ of biomass (Mitsch and Gosselink, 1986). Many consider *T. latifolia* to be a nuisance because it grows and reproduces rapidly, however, researchers have reported that this species can remove pollutants from stormwater wetlands very effectively

(Breed, 1993; Kadlec and Knight, 1996). *T. latifolia* grows in freshwater marshes, wet swales, streams, ponds, and along lake margins (Reddington, 1994). This species is found in all fifty states and is among the most common aquatic plants. *T. latifolia* can dominate large areas, especially where water levels fluctuate (USDA, 1999).

In the early spring, *T. latifolia* rapidly forms dense colonies that slow down stormwater-associated flows and allow particles to settle into the sediment (Stockdale, 1991). *T. latifolia* is also known to uptake such nutrients and heavy metals from stormwater wetlands as P, N, Cu, Ni, Zn, and Mg (Prentki et al., 1978; Breed, 1993; Taylor and Crowder, 1983). These pollutants are then stored in all parts of the plant, including the flower (Taylor and Crowder, 1983). These attributes of *T. latifolia* make it an excellent candidate for employment in stormwater constructed wetlands.

In this study we induced callus cell formation from *T. latifolia* leaf sections, and both male and female inflorescence spikes, and generated a growth curve for these cells in suspension culture. We report the initiation of callus cells from 100% of the female explant spikes that show a faster growth rate on semi-solid medium than reported in earlier studies. Previously, cattail callus cells were described by Zimmerman and Read (1986) who reported a low frequency of callus induction; later, Rogers et al., (1998) reported the development of *T. latifolia* callus cells that showed a very slow growth rate on Murashige and Skoog (MS) basal medium (Murashige and Skoog, 1962).

Material and Methods

T. latifolia leaves and inflorescence segments were collected on June 11, 1999 from Johnson Park on the Cook Campus of Rutgers University in New Brunswick, NJ. The developing inflorescence segments were totally enveloped in the foliage leaves. Before sterilization, the outer foliage leaves were removed and 20- to 30-cm long shoot sections containing the inflorescence were cut and rinsed with tap water. The shoot sections were rinsed in ethanol for 1 min then soaked in 0.5% chlorine containing 1% Tween-20® (Carolina Biological Supply Company) for 10 min followed by three sequential rinses for 1 min in sterile distilled water. The inflorescence sections were then cut in 5 mm thick cross-section pieces. The sterilization procedure for the leaves was the same as described for the inflorescence spikes. The explants were then placed into separate petri dishes containing either MS or Gamborgs B5 basal media supplemented with plant growth regulators (PGRs) dicamba or 2,4-D (2,4-Dichloro-phenoxy acetic acid) at 1, 2.5, or 5 mg●L^{-1} (Murashige and Skoog 1962; Gamborg et al., 1976). All media contained 0.8% agar, 3% sucrose, and were adjusted to a pH of 5.6 with 1N HCL or 1N KOH. The petri dishes were placed in the dark at 25 ± 1 ′C. Once callus cells were established, they were subcultured onto their original basal media with the addition of 0, 0.5, 1, 2.5, or 5 mg●L^{-1} BA (N^6-benzyladenine). The cells were subcultured every 4 weeks onto the same medium from which they were derived. Callus cells on petri dishes placed in 12 L/D

photoperiod formed shoots and were not used in this study.

Suspension cultures were generated from actively-growing undifferentiated callus cells isolated from 3-week-old stock cultures maintained on B5 basal medium supplemented with 5 mg●L^{-1} dicamba and 1 mg●L^{-1} BA. Callus tissue weighing 5 gm were placed into 125 mL Erlenmeyer flasks containing 60 mL of this basal medium without agar. Thirty-six flasks were set up for this experiment and placed on a rotary shaker operated at 110 rpm in the dark at 25 ± 1 $^{/}$C.

Once removed from the shaker, the flask contents were vacuum filtered to separate the callus cells from the medium. The cells were harvested, and their fresh weights were obtained to determine the net weight increase. Initially, the growth curve was intended to last 24 days, with 6 flasks removed from the shaker every 4 days. At day 16 of the growth curve, it was apparent that the cells would not reach the end of their exponential growth phase by day 24. Therefore, at day 20 adjustments were made in the number of flasks removed from the shaker. During this study, six flasks were harvested on days 4, 8, 12, and 16, while three flasks were harvested on days 20, 24, and 32.

Results

All three explant sources produced callus cells after 3 to 4 weeks in culture (Table 1), however, the immature female spikes were the superior explant source. Callus initiation from the female spike typically occurred between 7 and 10 days. They initially produced white, off-white, or beige callus cells on both MS and B5 media. After the third subculture the callus cells began to darken. The callus cells formed a hard, compact-to-loose mass. Callus initiation was from the inflorescence not from the stem. The callus mass formed only on the outer ring of the explant and not in the center (Fig. 1). This is similar to what was observed in Gramineae species (Straub et al., 1992). The callus cells did not form a complete spherical mass until separated from the explant and subcultured.

Callus cells were initiated from female spikes on both B5 and MS basal medium at all PGR concentrations tested (Table 1). Abundant callus growth from the female spike was obtained from MS basal medium supplemented with 5 mg●L^{-1} dicamba, which produced hard white, loose cells; B5 basal medium containing 2.5 mg●L^{-1} 2,4-D produced soft white, loose cells; and, B5 basal medium containing 5 mg●L^{-1} dicamba produced hard beige callus cells.

Callus initiation from immature male spikes was low (33%), and these callus cells grew very slowly. Only MS basal media induced callus formation from the male inflorescence spikes.

MS basal media supplemented with 5 mg●L^{-1} 2,4-D and MS basal media supplemented with 1 mg●L^{-1} dicamba induced hard, loose beige cells. The callus cells initiated from the male spikes were produced after 12 to14 days in culture. These cells were subcultured once, but eventually died.

In addition to the female and male inflorescence spikes, leaf tissue was the final explant source used in this study. Callus initiation from the leaf was slightly higher than the immature male spikes. The leaf produced callus cells at a rate of 66% and 33% on MS and B5 basal media,
respectively. Most of these cells were hard and white and grew at a very slow rate. A period of 14 to 21 days was required for callus initiation from the leaf tissue. Even after these cells formed, several months were required for them to reach a size sufficient for subculturing, we therefore determined that these cells were not suitable to establish a cell line.

Only callus cells derived from female spikes were suitable for subculturing. They were subcultured 4 weeks after initiation. Callus cells initiated on B5 basal media were bigger and had a more uniform growth pattern and color than the cells derived from MS basal media. All of the callus cells derived from the immature female spikes were subcultured onto B5 basal medium containing various PGR concentrations. After the third subculture, cells on B5 containing 1 and 5 mg●L^{-1} dicamba began to form roots (Fig. 2). To prevent root formation and stimulate callus growth, the cells were subcultured onto the same basal medium from which they

were derived with the addition of BA (Table 2). BA prevented root formation and accelerated the rate of callus growth. B5 basal medium supplemented with 5 mg●L^{-1} dicamba and 1 mg●L^{-1} BA was found to produce the largest mass of cells at the fastest rate (Fig. 3). This media was selected as the optimum growth medium.

The suspension cultures were established on B5 medium supplemented with 5 mg●L^{-1} dicamba and 1 mg●L^{-1} BA (Fig. 4). The lag phase lasted 4 days during which the cell mass increased by 2%. Subsequently, the cells entered their exponential growth phase, which lasted 20 days. During this time, the cell mass increased 150%. The doubling time for the cells in suspension culture was 16 days. Compared with other wetland plants grown in suspension culture, the growth rate of these cells was very slow. For example, *Distichilis spicata* (seashore saltgrass) exhibits a doubling time of approximately 40 hours, and *Catharanthus roseus* (madagascar periwinkle) doubles in 2 days (MacCarthy et al., 1980; Warren and Gould, 1982). The rapidly dividing *D. spicata* has a lag period of 2 days and an exponential growth phase of 7 days (Warren and Gould, 1982); *C. roseus* has a lag period of 4 days and an exponential growth phase of 14 days (MacCarthy et al., 1980).

Discussion

Using several tissue sources as explants, we have identified several new media formulations that can initiate *T. latifolia* callus cell formation. However, only the cells initiated from immature female spikes were suitable to establish a cell line because the cells formed a single layer that spread evenly across the petri plates, and the general color and appearance of the cells remained consistent after many subcultures, also, after adding BA to the media, no

apparent signs of embryogensis or organogenesis were visible. These characteristics presented a very stable cell line that can remain in culture indefinitely.

Zimmerman and Read (1986) concluded the type and concentration of PGR were critical for callus formation from *T. latifolia*. They reported callus initiation from male and female spikes used as explants. As the concentration of PGR changed, the frequency of callus formation varied. Zimmerman and Read (1986) reported callus initiation from 65% and 49% of the *T. latifolia* immature male and female inflorescence spikes respectively. The optimum basal medium developed in this present study induced callus formation from 100% of the female spikes used as explants (Table 1). In contrast, this present study indicates that explant source is the most important factor in callus formation; immature female spikes produced callus cells that could be used to establish a cell line when grown on several different media, while male inflorescence spikes and leaf sections did not produce viable cell lines on any of the media tested. In addition, the *T. latifolia* cells developed by Rogers et al., (1998) appeared to be about 7.5 mm in diameter at 9 weeks. In comparison, the *T. latifolia* cells developed in this study were more than 20 mm in diameter at 4 weeks. Therefore, to produce an abundant mass of cells relatively quickly, the culture media presented in this report is more suitable, because it generates callus cells at a higher frequency which had a faster growth rate than the callus cells reported by either Zimmerman and Read (1986), or Rogers et al., (1998).

It is unclear why the cells in suspension culture had an extended doubling time compared with other wetland plants, especially since the cells had a rapid growth rate on semisolid medium

when compared with the cattail cells formed by Rogers et al., (1998). In suspension culture, the cells developed in this study exhibited clearly distinguishable lag, exponential, and stationary phases of growth (Fig 4). However, because the cells had a normal growth cycle, the slow rate of growth was not a major concern.

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Table 1: Callus production from *T. latifolia* using various organs as sources of explants.

Plant Growth Regulator (PGR)		**B5 Basal Medium**			**MS Basal Medium**		
		Male	**Female**	**Leaf**	**Male**	**Female**	**Leaf**
1 mg•L^{-1} 2,4-D	**Callus Growth**	None	Moderate	Very little	None	Moderate	Slow
	Texture	------	Hard	Hard	------	Hard/Loose	Hard
	Color	------	White	Brown	------	White	White
	N	0	60/60	12/60	0	60/60	12/60
2.5 mg•L^{-1} 2,4-D	**Callus Growth**	None	Abundant	Very Little	None	Moderate	None
	Texture	------	Soft-Loose	Hard	------	Hard/Loose	------
	Color	------	White	White	------	White	------
	N	0	60/60	12/60	0	60/60	0
5 mg•L^{-1} 2,4-D	**Callus Growth**	None	Moderate	None	Very Little	Moderate	Very/Little
	Texture	------	Hard-Loose	------	Hard	Hard/Loose	Hard
	Color	------	White	------	Beige	Off-White	White
	N	0	60/60	0	60/60	60/60	48/60
Plant Growth Regulator (PGR)		**B5 Basal Medium**			**MS Basal Medium**		
		Male	**Female**	**Leaf**	**Male**	**Female**	**Leaf**
1 mg•L^{-1} Dicamba	**Callus Growth**	None	Moderate	None	Very Little	Moderate	None
	Texture	------	Hard-Loose	------	Hard/Loose	Hard	------
	Color	------	White	------	White	Off-White	------
	N	0	36/60	0	48/60	48/60	0

2.5 mg•L^{-1} Dicamba	**Callus Growth**	None	Moderate	None	None	Moderate	Very Little
	Texture	------	Hard	------	------	Hard	Hard
	Color	------	Beige	------	------	White	Beige
	N	0	48/60	0	0	60/60	24/60
5 mg•L^{-1} Dicamba	**Callus Growth**	None	Abundant	None	None	Abundant	Very Little
	Texture	------	Hard	------	------	Hard/Loose	Hard
	Color	------	Beige	------	------	White	White
	N	0	60/60	0	0	60/60	12/60

N=Number of explants forming callus cells/Number of explants placed in culture

Table 2: Maintenance of *T. latifolia* callus cells on B5 basal media supplemented with various hormone concentrations after 3 weeks in culture.

BA Concentration	**Auxin Concentration**	**Callus Growth[z]**
0 mg•L^{-1}	2.5 mg•L^{-1} 2,4-D	++
	5 mg•L^{-1} Dicamba	+++
0.5 mg•L^{-1}	2.5 mg•L^{-1} 2,4-D	+++
	5 mg•L^{-1} Dicamba	+++
1 mg•L^{-1}	2.5 mg•L^{-1} 2,4-D	+++
	5 mg•L^{-1} Dicamba	++++
2.5 mg•L^{-1}	2.5 mg•L^{-1} 2,4-D	+++
	5 mg•L^{-1} Dicamba	+++
5 mg•L^{-1}	2.5 mg•L^{-1} 2,4-D	+
	5 mg•L^{-1} Dicamba	+++

[z]+ = No callus growth; ++ = 1 - 10 mm callus; +++ = 11 - 20 mm callus; ++++ = > 20 mm callus

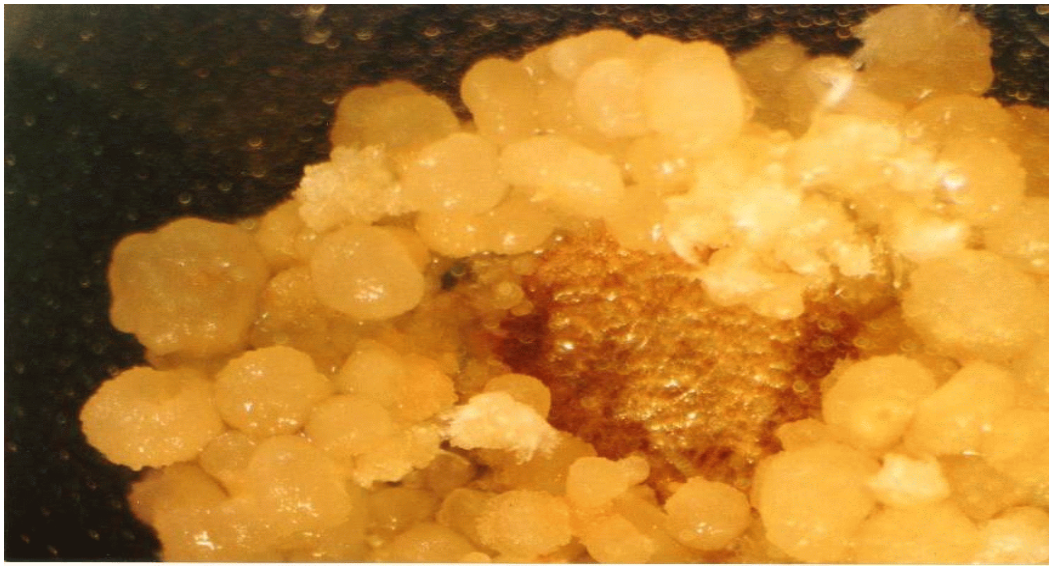

Figure 1: Callus initiation from female spikes.

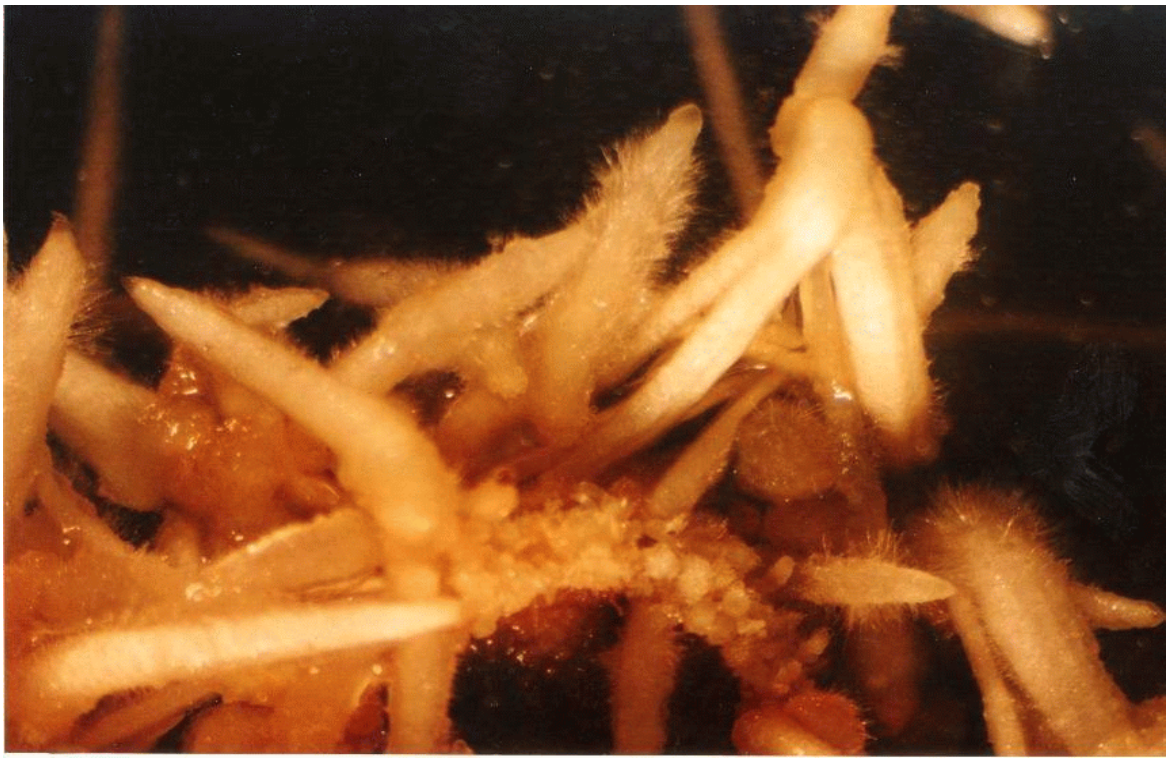

Figure 2: Root formation of from callus cells grown on B5 supplemented with 5 mg•L^{-1} dicamba and 1 mg•L BA^{-1}

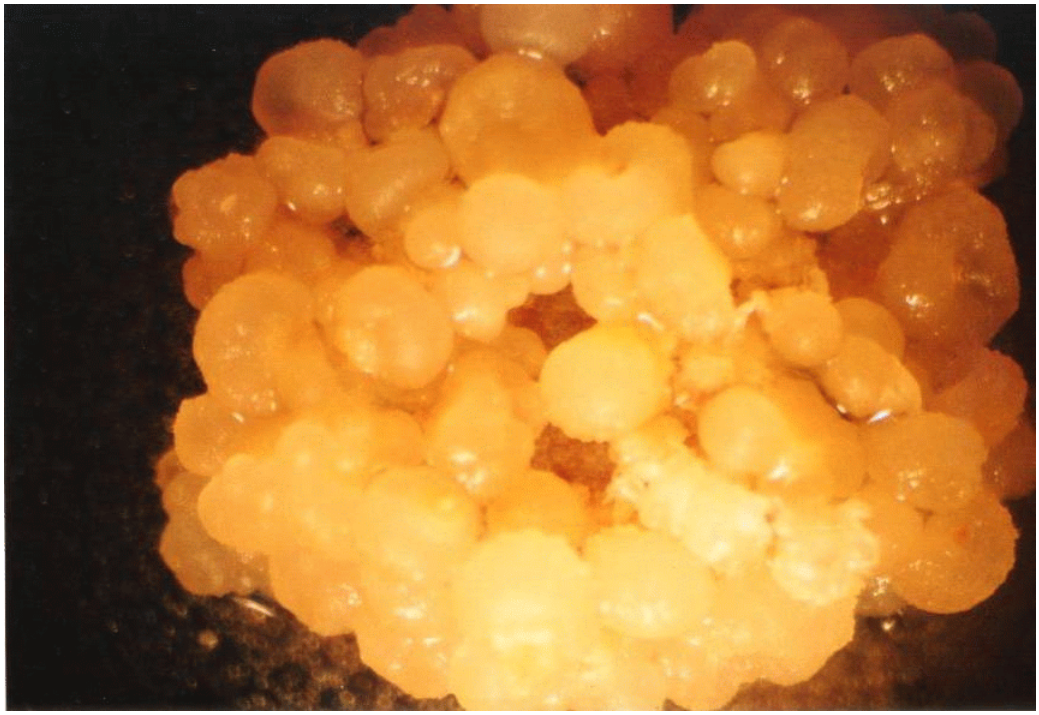

Figure 3: Callus cell formation on optimum basal medium.

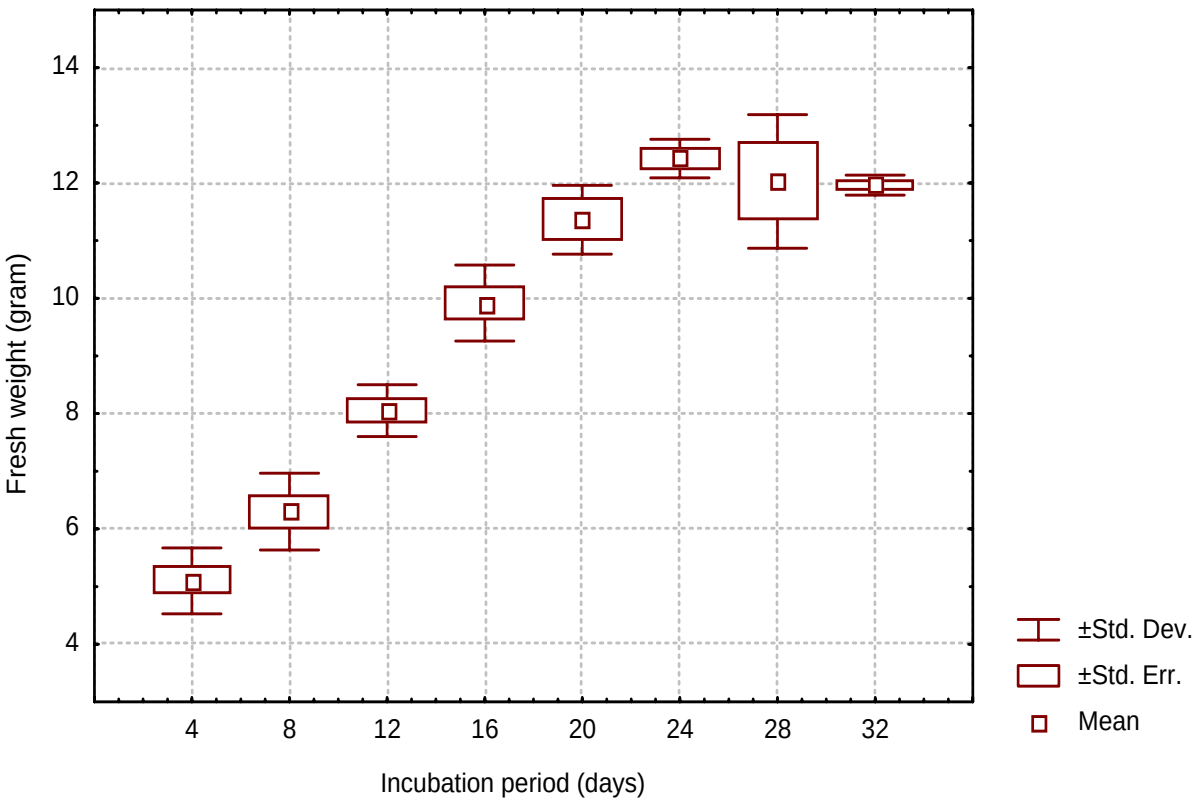

Figure 4: Suspension culture of *T. latifolia* cells grown in B5 medium supplemented with 5 mg •L^{-1} dicamba and 1 mg•L^{-1} BA.